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(54) **Dietary fiber, its process and physiologically active composition containing the same as an active ingredient.**

(57) A dietary fiber is disclosed which may be extracted from bran using an aqueous solvent at a temperature of 170 to 220°C. The fiber comprises, as its principal ingredient, water soluble and hard to digest polysaccharides rich in  $\beta$ -glucose. The fiber is useful in both dietary and pharmaceutical compositions, since it has tumour growth inhibiting activity, Lactobacillus bifidus proliferating activity, cholesterol metabolism improving activity and immunologic response enhancing activity. It is also quite safe to animals and humans.

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The present invention relates to a dietary fiber which can be obtained by extracting bran with an aqueous solvent at a high temperature and differs in chemical composition and physiological activity from a conventional extract of bran obtained with an aqueous solvent; its production; and a physiologically active composition which contains the dietary fiber as an active ingredient.

It has been known that some dietary fibers can be obtained by the extraction of bran with an aqueous solvent, but the low yield of the fibers obtainable by this conventional extraction method and the low physiological activity of the fibers obtained are amongst problems which have been encountered.

For instance, Japanese Patent Application Laid-Open (KOKAI) No. 63-165,325 (1988) discloses the extraction of degreased by-products, obtained in a polishing process of grains, such as rice bran, wheat bran or corn husk, using an aqueous solution of sodium hydroxide at room temperature. By this extraction only 4g of hemicellulose (B) was obtained from 100g of rice bran. Accordingly, the yield is extremely low and further, because of the extraction with an aqueous solution of NaOH, troublesome additional steps, such as neutralization and desalting, are necessary to purify the extract.

Japanese Patent Application Laid-Open (KOKAI) No. 64-62,303 (1989) discloses a method for extracting husks of grains or beans or a cellulose obtained from the husks using hot water at a temperature of 130 to 160°C, and purifying the extracts, but only a hemicellulose can be obtained by the method. This reference also discloses that when a husk of grain such as bran is extracted at a temperature no lower than 160°C, decomposition of the extract increases and is accompanied by a darkening of its colour. A low yield of useful substance is produced. Further, a substance extracted at a temperature of up to 160°C gives some deposition of hemicellulose during the purification step.

The present inventors have continuously studied for several years to obtain a useful and physiologically active substance from natural sources. For instance, they have succeeded in obtaining, by the extraction of Basidiomycetes with an aqueous solvent, an anti-tumour substance having a high molecular weight (see Japanese Patent Publication, 56-28,152 (1981)). Based on this experience they sought further to obtain in high yield hard to digest dietary fiber, which is a recent topic as a so-called functional foodstuff. As a result, they have found that a dietary fiber can be obtained in high yield if bran is extracted with an aqueous solvent at a temperature as high as 170 to 220°C, contrary to common knowledge among those skilled in the art.

They also found that in addition to the so-called dietary fiber's properties, such as promotion of digestion, absorption of nutrients and excretion of waste, and proliferation of *Lactobacillus bifidus* within the large intestine, the fiber possesses very useful physiological activities such as anti-tumour, immunological and cholesterol metabolism improving activities.

The present invention is based on these findings.

Figure 1 illustrates an infrared absorption spectrum of the sample 2 obtained in Example 1.

The present invention provides a dietary fiber which is obtainable by the extraction of bran with an aqueous solvent at a temperature of 170°C to 220°C. The fiber includes water-soluble and hard-to-digest polysaccharides of which the glucose component has a molar ratio of  $\alpha$ -bound glucose to  $\beta$ -bound glucose of from 1:40 to 1:80.

The present invention further provides a dietary fiber having antitumour, cholesterol metabolism improving and immunologic activities in addition to usual physiological activities of dietary fiber and a physiologically active composition which contains the fiber as an active ingredient.

The present invention also provides a process for producing dietary fiber, which process comprises extracting bran with an aqueous solvent at a temperature of 170 to 220°C.

The term "bran" in the present invention means whole by-products of processing grains which has been mainly used as a feed for livestock. As representative grains, wheat, barley, rice and corn can be exemplified and rye and oat are also included.

The "dietary fiber" in the present invention means the polysaccharides which remain unaltered, neither digested nor absorbed, in a digestive tract and excreted as far as it is in the original state.

By "principal ingredient" is meant an ingredient present in an amount greater than 50% by weight, typically greater than 60%, preferably greater than 70%, for example 76% or above.

The dietary fiber of the present invention (hereinafter referred to as "the present substance") can be obtained by extracting bran with an aqueous solvent and has following properties:

(a) The average molecular weight determined by the GPC (gel permeation chromatography)-Lalls method is  $5 \times 10^2$  to  $1 \times 10^5$ , preferably  $5 \times 10^3$  to  $5 \times 10^4$ .

(b) It has peaks around  $3300 \text{ cm}^{-1}$  and  $1650 \text{ cm}^{-1}$  in an infrared absorption spectrum.

(c) The specific rotation is

$$[\alpha]_D^{25} = +138 + -145^\circ \quad (c = 0.25, \text{H}_2\text{O}).$$

(d) The sugar content determined by a color reaction of phenol-sulfuric acid method is 95.3 to 99.0% by weight and the protein content determined by a color reaction of Lowry-Folin method is 0.1 to 3.0% by weight.

(e) The elementary analysis indicates 35.3 to 39.3% carbon, 4.2 to 6.5% hydrogen and 0.05 to 0.9% nitrogen, all by weight.

(f) It is highly soluble to water, and hardly soluble in chloroform, benzene, ethanol and ether.

(g) It has no clear melting point and starts to decompose, to become black and to carbonize gradually at a temperature around 250°C.

(h) The sugar component comprises at least glucose, xylose and galactose, preferably contains 88 mol% or more glucose, and more preferably contains 88.2 to 95.4 mol% glucose, 3.7 to 7.7 mol% xylose, 0.1 to 1.2 mol% galactose, 1.0 mol% or less fucose and 1.1 mol% or less arabinose.

(i) The composite amino acids comprises at least aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cysteine, valine, isoleucine, leucine, tyrosine, phenylalanine, hydroxylysine, lysine, histidine and arginine.

Further, the mol% of individual amino acids is preferably in the following range, all in mol%:

aspartic acid, 1.8 to 4.8; threonine, 2.4 to 4.0; serine, 6.0 to 8.7; glutamic acid, 27.1 to 33.0; proline, 9.3 to 12.8; glycine, 12.7 to 14.6; alanine, 6.6 to 8.8; cysteine, 0.1 to 0.3; valine, 3.7 to 5.8; isoleucine, 2.5 to 4.2; leucine, 4.4 to 5.8; tyrosine, 1.2 to 2.5; phenylalanine, 3.4 to 4.8; hydroxylysine, 0.9 to 2.5; lysine, 1.3 to 2.4; histidine, 0.9 to 1.9; and arginine, 0.3 to 1.8.

(j) The molar ratio between  $\alpha$ - and  $\beta$ -bound glucoses in the glucose component is 1:40 to 1:80.

(k) The molar concentration of 1,4-glucose bond is 85 to 90% and that of 1,3-glucose bond is 4 to 10%.

(l) The molar concentration ratios of methylated sugars obtained by hydrolysis of the present substance are in the following ranges:

	<u>Ratio</u>
2, 3, 4 6-Tetramethylglucose;	1.0
2, 4, 6-Trimethylglucose;	0.55 to 0.93
2, 3, 6-Trimethylglucose;	7.96 to 17.94
2, 6-Dimethylglucose;	0.12 to 0.33
3, 6-Dimethylglucose; and	0.03 to 0.11
2, 3-Dimethylglucose;	0.48 to 0.70

(m) The analytical result of the present substance by the Southgate method is as follows:

	<u>% by weight</u>
Water-soluble and hardly digestible polysaccharides;	78 to 96
Cellulose;	1.0 to 5.7
Hemicellulose	1.7 to 15
Lignin	1.3 to 3.3

The Southgate method is a quantitative analytical method of a total dietary fiber content and in addition to cellulose, hemicellulose and lignin which are components of cell wall, macromolecules such as water-soluble and hardly digestible polysaccharides can be determined [see "Determination of Carbohydrates in Foods. II. Unavailable Carbohydrates", J. Sci. Food Agric., 20, 331 (1969)].

Description will be made now for the production method of the present substance, which is a part of the present invention. It is a method for obtaining a dietary fiber of the present invention in a high yield by extracting

a bran, a starting material, with an aqueous solvent at 170 to 220°C or preferably at 180 to 200°C. The extraction time can be adjusted appropriately in accordance with the thermal condition described above, but generally it is preferably 5 to 60 minutes and more preferably 10 to 30 minutes. If the temperature is lower than 170°C or if the time is less than 5 minutes even when the temperature is maintained not lower than 170°C, the extraction does not proceed sufficiently. If the temperature is higher than 220°C, or if the time exceeds 60 minutes even when the temperature is maintained not higher than 220°C, the extracted substance suffers from decomposition.

This extraction process can be repeated a number of times if the conditions are maintained in the range as specified above.

The aqueous solvent in the present invention is at least one selected from the group consisting of water, water-soluble organic solvents, aqueous solutions containing a small amount of acid or base. The concentration of the aqueous solution containing a small amount of acid or base should be determined in accordance with the kind of the acid or base, but generally an aqueous solution having a concentration of 10% by weight or less can preferably be used. Examples of organic solvents include methanol, ethanol, isopropyl alcohol, and the like. Examples of acids include hydrochloric acid, sulfuric acid, acetic acid, and the like, and of bases, include ammonia, sodium hydroxide, potassium hydroxide, sodium carbonate and the like.

Among these aqueous solvents, water is most preferable when the effect of the solvent on the quality of the extracted substance, and the difficulty involved in purifying the extract are taken into consideration.

The extracted solution, after removing insoluble substances, is neutralized and purified when necessary. A purification of the extracted solution is to remove low molecular weight substances with at least one method selected from the group consisting of salting-out, dialysis, ultrafiltration, reverse osmosis, gel filtration and precipitation by an organic solvent. The molecular weight of the low molecular weight substances to be removed varies according to the type of bran used, extraction conditions, a kind of the aqueous solvent and a necessary quality of the product, therefore it can not be determined simply. However, when wheat bran and water is used as a material and an aqueous solvent, substance having a molecular weight not higher than 400 are preferably removed because it would bring good result in decoloration and deodorization of the present substance.

The dietary fiber after extraction or purification can be used as a solution or a solid after being dried. Also, water soluble and hard to digest polysaccharides can be isolated and used singly. The water soluble and hard to digest polysaccharides in the present invention means substances determined by the Southgate method which will be described in detail later in the Examples.

A bran, a raw material, can be extracted as it is, but it may also be treated chemically, enzymatically or physically prior to the extraction in order to facilitate an extraction of a specific component of the bran.

Further, the present substance has the following physiological activities.

#### 1. Antitumor activity:

When the present substance is injected intraperitoneally to the Sarcoma-180 transplanted mouse, the inhibition of tumor growth is preferable 50% or more, or more preferably 60% or more.

#### 2. Proliferation activity of Lactobacillus bifidus;

When the present substance is administered orally to a mouse, proliferation ratio of Lactobacillus bifidus in its feces is 10 fold or more than that of the control.

#### 3. Activity reducing an accumulation of cholesterol:

When the present substance is administered orally to a mouse suffering from hypercholesterolemia, the cholesterol level in blood is reduced by about 20% and the level in liver, by about 30%.

#### 4. Activity stimulating immunologic response:

The present substance was added together with phytohemagglutinin and chlorambucil to monocytes obtained from human peripheral blood and the effect of the present substance on the lymphocytes was studied based on an uptake of <sup>3</sup>H-thymidine by the lymphocytes. The present substance significantly improves an activity of the lymphocytes which has been suppressed by chlorambucil.

#### 5. Acute toxicity with a large dose of the present substance:

The present substance was administered orally at a dose of 5 g/kg of body weight to a mouse, and its toxicity

was studied. Weight reduction was not observed as compared with the control group and no death occurred. From the above, it is expected that the LD<sub>50</sub> as the oral administration acute toxicity of the present substance is larger than 5,000 mg/kg of body weight.

The methods used for determining above activities and their results will be described in detail in Examples 3 to 8.

As is obvious from the above, the present substance is not only remarkably low toxic but also possesses a variety of useful physiological activities. Accordingly, as is described below, the present substance is beneficial if it is used as an ingredient of a pharmaceutical composition and a dietary composition.

As a pharmaceutical composition, it can be used, judging from the activities described before, as an anti-tumor drugs, growth-stimulants of *Lactobacillus bifidus* in intestine, a cholesterol metabolism improving agent and a stimulant of immunologic reaction. In these pharmaceutical composition, the present substance can be administered orally or parenterally. It can also be used in any conventional dosage form, such as syrup, pill, powder, granule, tablet, capsule, suppository, cream, ointment or spray. In these drug forms, it can be used alone or together with bases such as activators, bonding agents, disintegrating agents, lustering agents, coloring agents or fillers.

The present substance can be used, making better use of the above activities, as a dietary compositions having anti-tumor activity, growth stimulating activity of *Lactobacillus bifidus*, cholesterol metabolism improving activity and/or immunology enhancing activity.

The dietary composition, for example, includes salted bean paste (miso), soy bean curd (tofu), retort foods, frozen foods, bread, pie, confections stuffed with sweetened bean paste, fish meat product such as salted, ground and whitened fish meat (kamaboko), baked, ground fish meat (chikuwa), meat products such as hamburger, meat balls, etc., powdered soup, powdered fat, powdered extract, powdered seasonings, powdered sweeteners, candies, drops, boiled and salted kelp (tsukudani), grated cheese, sauce, ketchup, dressing, mayonaise, sauce for grilled meat, biscuits, cookies, wheat noodles (udon), buckwheat noodles (soba) cream, spread, based and dried rice cake (okaki), baked rice cake (senbei), and so on. It is needless to say that the present substance can also be added to general foodstuffs other than listed above in order to give them its unique properties.

The present substance, as a component of a pharmaceutical composition or a dietary composition, can be used as a prophylaxis or a treating agent for the diseases described above. When used as a component of a pharmaceutical composition, it can be administered in a conventional dosage form preferably at a dosage of 0.01 mg/kg body weight to 1,000 mg/kg body weight and more preferably at a dosage of 0.1 to 500 mg/kg body weight, once to several times daily. When used as a dietary composition, it can be given by itself or as a component of a foodstuff or beverage generally at a preferable dosage of 0.01 to 10,000 mg/kg body weight and at more preferable dosage of 0.1 to 1,000 mg/kg body weight daily for an adult.

### EXAMPLES

The present invention will be explained below more specifically based on the examples. However, the scope of the present invention is not necessarily limited to the examples describe below.

#### Example 1:

##### (1) Preparation of the present substance.

A mixture of 100 g of a wheat bran commercially available and 3 l of water were placed in an autoclave and heated at 190°C for 20 minutes for extraction. The unextracted substance was filtered out, about 4 volume of ethanol was added to one volume of the filtrate and the mixture was kept at least overnight in a dark and cool place, and obtained 22.5 g of polysaccharide precipitate. The precipitate, after being dissolved in water, was dialyzed in cold water for 3 days using a visking tube (manufactured by Union Carbide Corp.), and freeze-dried to give 19.4 g of white dietary fiber being tasteless and odorless (the yield being 19.4%) [Sample 1].

Alternatively, only changing the extraction condition, another dietary fibers were obtained. The extraction was performed at 180°C for 20 minutes [Sample 2] and at 170°C for 20 minutes [Sample 3]. The yield was 26.2% for the sample 2 and 27.5% for the sample 3.

##### (2) Preparation of the present substance.

A mixture of 600 g of a wheat bran commercially available and 20 l of water were placed in an autoclave and heated at 180°C for 15 minutes for extraction. The unextracted substance was filtered out by continuous

centrifugation, the filtrate was passed through a column filled with activated carbons and alumina to remove low molecular weight substances and the resultant solution was passed through a ultrafiltration membrane (BI-FU-T2/A manufactured by PCI Co.) to obtain components having a molecular weight not lower than 5,000. The filtrate was freeze-dried and obtained 150 g of white dietary fiber being tasteless and odorless (the yield being 25%) [Sample 4].

#### Comparison Example 1:

A mixture of 100 g of a wheat bran commercially available and 3 l of water were placed in an autoclave and heated at 160°C for 20 minutes. The extracted solution was treated in the same manner as Example 1 and obtained 30.5 g of dietary fiber [Sample 5].

Alternatively, only changing the extraction condition, a dietary fiber was obtained. The extraction was performed at 150°C for 20 minutes [Sample 6] and at 140°C for 20 minutes [Sample 7]. The yield was 28.5% for the sample 6 and 26.4% for the sample 7. In addition, the extraction was performed at 100°C for 20 minutes and obtained a dietary fiber [Sample 8].

#### Example 2:

The dietary fibers, samples 1 to 7, obtained in Example 1 and Comparative Example 1 was analyzed to obtain the following data.

##### (1) Molecular Weight:

With Waters gas permeable chromatography, as one of GPC-Lalls method, a molecular weight of a solute and content of its fractions were calculated from its scattering intensity difference and refractive index difference of light passed through the solution dissolving the molecular chain of the sample and divided in size. Calculated molecular weight was the weight average molecular weight.

##### (2) Infrared absorption spectrum:

The infrared absorption photometry of the samples were taken with a spectrophotometer (A-202, manufactured by NIHON BUNKO Co.) applying KBr tablet method. Among the spectra of the samples 1 to 7, a spectrum of the sample 2 is shown in Figure 1 as the representative.

##### (3) Optical rotation:

An aqueous solution containing 0.25% present substance (samples 1 to 7) was subjected to a polarimetric analysis with an automatic spectropolarimeter (DIP-360, manufactured by NIHON BUNKO Co.) and determined the specific rotations of the samples.

##### (4) Sugar content:

The sugar content of the present substance calculated as glucose was determined based on a color reaction of the phenol-sulfuric acid method with a spectrophotometer in a range of ultraviolet to visible light (UV-160A; manufactured by SHIMAZU SEISAKUSHO).

##### (5) Protein content:

The protein content of the present substance calculated as bovine serum albumin was determined based on a color reaction of the Lowry-Folin method with a spectrophotometer in a range of ultraviolet to visible light (UV-160A).

##### (6) Elementary analysis:

A decomposed gas from the present substance was analyzed with a TCD detector in an automatic elementary analyzer (MT3; manufactured by YANAGIMOTO SEISAKUSHO).

## (7) Solubility:

The solubility was measured according to the description in Japan Pharmacopoeia. The present substance is readily soluble to water but hardly soluble to chloroform, benzene, ethanol and ether.

## (8) Composition of the sugar components:

The sugar components of the present substance were separated with an anion exchange resin in Bio-Liquid Chromatography (Bio-LC; manufactured by DIONEX Co.) and its composition was identified by a pulsed-and-metric method.

## (9) Composition of the amino acid components:

The present substance, after being hydrolyzed, was analyzed with an amino acid analyzer (073 Type; HITACHI SEISAKUSHO), to give amino acid composition.

## (10) Enzymatic analysis:

The present substance was hydrolyzed and the molar ratio between  $\alpha$ - and  $\beta$ -glucoses was determined with  $\alpha$ -1,4- and  $\beta$ -1,4-glucosidases to have a ratio between  $\alpha$ - and  $\beta$ -glucose bonds.

## (11) Sugar bond:

The present substance was, after methylation, hydrolyzed, and the resultant partially methylated sugars were acetylated and analyzed with a gas chromatograph (GC-14A; manufactured by SHIMAZU SEISAKUSHO) and a mass spectrometer (JMS DX-303, NIHON DENSHI Co.), thereby obtained identification and determination of the methylated sugars.

## (12) Melting point:

The melting point of the present substance was measured by the DSC (Differential Scanning Calorimeter) method, but all the samples did not show clear melting point but turned black at around 250°C and carbonized.

The results of the samples 1 to 7 obtained by the above analytical methods (1) to (12) are summarized in Table 2.

## (13) Analysis of dietary fibers by Southgate method:

The content of water soluble and hardly digestible polysaccharide, cellulose, hemicellulose and lignin in the present substance was determined by the Southgate method, as follows:

To heated 85% ethanol, 100 g of each of samples 1 to 7 was placed to remove free sugars, and the solution was placed in a centrifuging tube of 50 ml capacity. A 60 ml of distilled water was added to the solution, and the mixture was heated in a boiling water bath to make the contained starch pasty and after cooled, 0.3 ml of 2M acetic acid salt buffer solution (pH 4.6) and 1.5 ml of 10% takadiastase solution were added and the mixture was maintained at 37°C for 18 hours to decompose starch. Then, 30 ml of ethanol was added to the solution, and the mixture was placed overnight in a cool place and centrifuged to remove glucose derived from starch. The remained solution was dried and obtained about 30 g of high molecular weight dietary fiber hardly digestible.

Then, to 100 mg of the hardly digestible dietary fiber obtained by the enzymatic treatment, 3 ml of distilled water was added and the mixture was heated on a boiling water bath to remove ethanol. To the obtained solution 10 ml of heated distilled water was further added and the mixture was heated for 20 minutes on a boiling water bath and centrifuged to divide into two fraction, that are, a solution containing hot water soluble substance and a solid which is insoluble in hot water. The former fraction contained the water soluble and hardly digestible polysaccharide and the latter fraction contained hemicellulose, cellulose and lignin. The hot water soluble fraction was cooled, 4 volume of ethanol was added to one volume of the fraction and the mixture was centrifuged. 10 ml of 1 N sulfuric acid was added to the residue thus centrifuged and the mixture was heated at 100°C for 2.5 hours. The hydrolyzed product was analyzed by the phenol-sulfuric acid method and determined the water soluble and hardly digestible polysaccharide.

Then, to isolate hemicellulose from the mixture of hemicellulose, cellulose and lignin in the hot water insol-

uble fraction, 10 ml of 1 N sulfuric acid was added to the fraction in a centrifuging tube. The mixture was heated at 100°C for 2.5 hours for hydrolysis and centrifuged. The supernatant of the fraction soluble to a diluted acid was used to determine the water insoluble hemicellulose component.

5 To the residue obtained by removing the dilute acid soluble fraction, 10 ml of cold 72% sulfuric acid was added and the mixture was left to stand at 4°C for 48 hours. The mixture was filtered under reduced pressure through a glass fiber filter placed on a Gooch crucible. The filtrate, the fraction soluble to cold 72% sulfuric acid, was used to determine the cellulose component.

The residue in the Gooch crucible was incinerated by heating at 500°C for 3 hours. The weight of residue subtracted by an ash weight was taken as the lignin content.

10 Weights of the water soluble and hardly digestible polysaccharide, hemicellulose, cellulose and lignin under variable extracting conditions are summarized in Table 1. As is indicated in the table, when the extraction temperature is 170°C or higher, the dietary fiber having the polysaccharide as the

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Table 1

Number	Conditions of Extraction		Yield of Dietry Fiber (%)	Analytical Result of Dietry Fiber by Southgate Method (%)			
	Temp. (°C)	Time (Minute)		Polysaccharide*1)	Cellulose	Hemicellulose	Lignin
Example 1	190	20	19.4	94.4	1.6	1.8	2.2
Example 2	180	20	26.2	92.8	1.7	3.2	2.3
Example 3	170	20	27.5	78.2	4.1	14.8	2.9
Example 4	180	15	25.0	84.3	4.6	8.5	2.6
Com. Ex. 1 *2)	160	20	30.5	23.6	14.2	59.5	2.7
Com. Ex. 2 *2)	150	20	28.5	22.5	15.9	58.1	3.5
Com. Ex. 3 *2)	140	20	26.4	21.6	19.3	55.2	3.9

\*1) This polysaccharide means a water-soluble polysaccharide which is hardly digestable.

\*2) Com. Ex. means Comparative Example.

Table 2-I

No. *4)	IR Absorption		$[\alpha]_D^{25}$ *1)	Elementary Analysis			Amount of Sugar *2) (weight %)	Amount of Protein *3) (weight %)	Molecular Weight
	3300 cm <sup>-1</sup>	1650 cm <sup>-1</sup>		C (%)	H (%)	N (%)			
Exam. 1	+	+	+141°	35.4	5.6	0.2	99.0	0.5	18,000
Exam. 2	+	+	+142°	37.3	5.7	0.3	96.8	1.8	23,000
Exam. 3	+	+	+143°	36.8	5.7	0.6	96.8	2.2	84,000
Exam. 4	+	+	+144°	36.2	5.5	0.6	97.0	2.0	38,000
Com. Ex. 1	+	+	+147°	35.0	6.8	1.6	95.0	3.1	≥ 100,000
Com. Ex. 2	+	+	+146°	34.4	6.7	2.2	94.4	3.3	≥ 100,000
Com. Ex. 3	+	+	+146°	32.8	6.8	2.1	94.4	3.4	≥ 100,000

\*1)

Specific rotatory power measured at C = 0.25, H<sub>2</sub>O

\*2)

Measured with phenol-sulfuric acid method

\*3)

Measured with Lowry-Folin method

\*4)

Exam. means Example and Com. Ex. means Comparative Example

Table 2 - II

Number	Sugar Constituent (Mol %)					$\alpha$ -Binding $\beta$ -Binding in Molar Ratio of Glucose Binding
	Glucose	Xylose	Galactose	Arabinose	Fucose	
Exam. 1	94.0	5.6	0.2	0.1	0.1	1/80
Exam. 2	92.3	7.3	0.2	0.1	0.1	1/50
Exam. 3	89.4	7.7	1.0	1.0	0.9	1/40
Exam. 4	90.1	7.5	1.2	0.8	0.4	1/50
Com. Ex. 1	87.7	8.8	1.3	1.1	1.1	1/30
Com. Ex. 2	85.3	11.2	1.3	1.1	1.1	1/20
Com. Ex. 3	82.8	13.7	1.3	1.1	1.1	1/20

Table 2 - III

No.	Amino Acid Constitution (Mol %)																
	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Ile	Leu	Tyr	Phe	Hyl	Lys	His	Arg
Exam. 1	2.7	2.9	7.2	32.7	10.4	13.2	7.2	0.2	4.2	3.6	4.6	1.8	3.8	1.9	1.8	1.4	0.4
Exam. 2	2.9	3.7	7.7	30.1	10.8	13.7	7.6	0.1	4.7	3.2	5.4	1.5	3.8	1.5	1.4	1.2	0.7
Exam. 3	4.2	3.7	7.2	31.2	10.6	13.4	7.4	0.1	4.0	3.0	5.5	1.3	3.3	0.9	1.5	1.5	1.2
Exam. 4	3.5	4.0	7.5	29.7	10.2	12.8	8.0	0.1	4.4	3.4	5.2	2.0	3.5	1.2	1.5	1.2	1.8
Com. Ex. 1	4.9	4.1	5.1	33.3	12.9	10.7	8.9	0.4	2.5	2.3	5.9	1.0	2.5	0.4	1.2	2.0	1.9
Com. Ex. 2	4.9	4.1	5.0	33.8	12.9	9.5	8.9	0.4	2.6	2.2	6.0	1.0	2.2	0.8	1.2	2.1	2.4
Com. Ex. 3	5.0	4.2	5.8	33.7	13.0	8.3	9.0	0.4	2.8	2.4	5.9	1.1	2.3	0.8	1.1	2.0	2.2

Asp = Aspartic acid; Thr = threonine; Ser = serine; Glu = glutamic acid; Pro = proline; Gly = glycine;

Ala = alanine; Cys = cystine; Val = valine; Ile = isoleucine; Leu = leucine; Tyr = tyrosine; Phe = phenylalanine

Hyl = hydroxylysine; Lys = lysine; His = histidine; Arg = arginine

Table 2 - IV

Number	Methylated Sugar Content (Mol %) *1)					
	2,3,4,6-(CH <sub>3</sub> ) <sub>4</sub> GLC	2,4,6-(CH <sub>3</sub> ) <sub>3</sub> GLC	2,3,6-(CH <sub>3</sub> ) <sub>3</sub> GLC	2,6-(CH <sub>3</sub> ) <sub>2</sub> GLC	3,6-(CH <sub>3</sub> ) <sub>2</sub> GLC	2,3-(CH <sub>3</sub> ) <sub>2</sub> GLC
Exam. 1	1.0	0.60	8.52	0.16	0.04	0.58
Exam. 2	1.0	0.62	11.96	0.22	0.08	0.68
Exam. 3	1.0	0.58	13.12	0.29	0.09	0.62
Exam. 4	1.0	0.60	10.08	0.27	0.07	0.62
Com. Ex. 1	1.0	0.54	18.42	0.34	0.12	0.71
Com. Ex. 2	1.0	0.52	22.14	0.36	0.13	0.74
Com. Ex. 3	1.0	0.52	25.35	0.36	0.12	0.72

\*1) GLC means glucose.

main component is obtained, while when the temperature is lowered than 160°C, a dietary fiber largely composed of hemicellulose is obtained.

As is apparent from Tables 1 and 2, the dietary fiber of the present invention is clearly different from the fiber extracted by water at a temperature below 160°C.

### Example 3:

To a group of ICR-JCL mice (8 mice/group) subcutaneously transplanted with 10<sup>6</sup> Sarcoma-180, the present substance (sample 2) dissolved in 0.1% physiological saline solution was intraperitoneally injected with a dosage amount of 10 mg/kg body weight 10 times every other day starting from 2 days after the transplantation. Two days after the completion of the injection, the tumors were weighed. The control mice were injected only a physiological saline and their tumors were also weighed in the same manner. From these results, the inhibitory effect on tumor growth was calculated from the following equation and the result is shown in Table 3.

$$\text{I. R. (\%)} = \left[ \frac{\text{Average Tumor weight of Control Group} - \text{Average Tumor weight of Testing Group}}{\text{Average Tumor weight of Control Group}} \right] \times 100$$

Table 3: Antitumor Activity of the Present Substance

Sample	Average Weight of Tumor (mg) ( $\bar{X} \pm \text{S.D.}$ )	Inhibiting Ratio I. R. (%)
Present Substance (Sample No. 2)	797.5 $\pm$ 755.8	84.9
Control	5,282.2 $\pm$ 6,849.4	----

### Example 4:

A 5% aqueous solutions of the present substances prepared in Example 1 were prepared and 5% aqueous solution of fructo-oligosaccharide commercially available was also prepared as a comparison.

The above preparations are given forcibly orally to groups (10 mice/group; 3 groups) of C<sub>3</sub>H/He female mice (8 weeks of age) at a daily dose of 0.5 g/kg body weight, as a solid, for 21 days.

For the control group, a same amount of physiological saline was forcibly given orally to mice for the same period. Before and after the tests, feces of the mice under study were collected. The feces were put into 100 volume of anerobic diluting solution (phosphate buffer) and milled the feces to powder. A 0.1 ml aliquot of these mixture were respectively coated on a surface of B.S medium and incubated anerobically (an anerobic glove box method) at 37°C for 1 to 5 days. After the cultivations were over, number of Lactobacillus bifidus on each medium was counted. The results are shown in Table 4. Each figure in the table indicates an average numbers obtained by ten animals.

Table 4: Growth Rate of Lactobacillus bifidus by the Present Substance

Sample	No. of the Bacterium/g of Feces		(B)/(A)
	Before Administration (A)	21st Day After Administration (B)	
Sample 2	$3.8 \times 10^4$	$4.6 \times 10^5$	12.1
Sample 1	$3.8 \times 10^4$	$5.1 \times 10^5$	13.4
Sample 3	$3.8 \times 10^4$	$4.8 \times 10^5$	12.6
Fructo-oligosaccharide	$3.9 \times 10^4$	$3.9 \times 10^4$	1.0
Control	$4.1 \times 10^4$	$3.7 \times 10^4$	0.9

Example 5:

C<sub>3</sub>H/He mice in four groups (28 mice/group) were freely given a feed which induce in mouse hypercholesterolemia experimentally and at the same time the present substance prepared in Example 1 were forced to take orally at a dose of 500 mg/kg body weight daily.

Mice in a control group were forced to take orally 500 mg/kg of physiological saline daily instead of the present substance. The result is given in Table 5. The result indicates that the present substance is significantly reduce accumulation of cholesterol in blood and liver.

Table 5: Reducing Effect on Cholesterol Accumulation

Sample	Cholesterol Content (mg/ml)	
	In Blood	In Liver
Present Substance (Sample 2)	135	38.3
Present Substance (Sample 1)	130	37.4
Present Substance (Sample 3)	140	40.4
Control	167	58.1

Example 6:

Phytohemagglutinin, which is one of representative mitogens, and chlorambucil (hereinafter referred to as "CBL"), which is a suppressor of lymphocyte and is a antitumor agent, were allowed to act upon monocytes (one of lymphocytes) obtained from human peripheral blood. At the same time, the present substances prep-

ared in Example 1 and dietary fibers prepared in Comparative Example 1 were respectively given in an amount of  $10^{-2}$   $\mu$ g to each of the above mixture. After incubation of a certain period, the uptakes of  $^3\text{H}$ -thymidine by lymphocytes were determined and the effect of the present substance on the human lymphocytes were evaluated.

As a positive control, lentinan, an antitumor agent in an immunologic therapy, was used instead of dietary fibers. The results are given in Table 6.

The results indicate that the present substance is effective in restoring the activity of human lymphocytes which has been significantly suppressed, while dietary fiber prepared with an extraction at a temperature below  $160^\circ\text{C}$  does not show the effect.

Table 6. Relation between Extraction Temperature and Immunology Activation (in vitro)

Sample	Blastogenesis of Monocyte	
	Uptake of $^3\text{H}$ -Thymidine (cpm)	Activity
Present Substance (Sample 2) + CBL 2.5 $\mu$ g/ml	$138,775 \pm 12,700^{**}$	○
Present Substance (Sample 1) + CBL 2.5 $\mu$ g/m	$148,775 \pm 12,700^{**}$	○
Present Substance (Sample 3) + CBL 2.5 $\mu$ g/ml	$135,226 \pm 10,883^{**}$	○
Comparative Example (Sample 5) + CBL 2.5 $\mu$ g/ml	$92,175 \pm 6,802$	X
Comparative Example (Sample 7) + CBL 2.5 $\mu$ g/ml	$73,901 \pm 2,351$	X
Comparative Example (Sample 8) + CBL 2.5 $\mu$ g/ml	$91,865 \pm 2,157$	X
Lentinan	$93,620 \pm 6,651$	X
Control	$88,832 \pm 12,700$	--

$^{**}$ :  $p < 0.01$ : ○: Active: X: Inactive

#### Example 7:

Groups of 10 normal C57BL/6.J female mice were forced to take orally CBL at 10 mg/kg body weight and at the same time, the present substances prepared in Example 1 and dissolved in physiological saline at a dose of 250 mg/kg body weight were given freely for 5 days, daily. After the tests were over, spleen of each mouse was extirpated and number of lymphocytes in it was counted. Then, suspensions prepared at a concentration



of  $2.5 \times 10^6$  lymphocytes/ml were respectively placed on a each well of 96-well microplate with phytohemagglutinin and incubated for a certain period. After the incubations the uptake of  $^3\text{H}$ -thymidine by the lymphocyte was measured and the effect of an oral administration of the present substance on the splenic lymphocytes in mouse was studied.

To a control group, physiological saline without any dietary fiber was given freely and the effect on the splenic lymphocytes in mouse was studied in the same manner as above. The result is given in Table 7.

The result indicates that ingestion of the present substance is significantly effective even in recovering an abruptly reduced activity of mouse splenic lymphocytes and in maintaining their blastomogenous activity.

Table 7:

Effect of The Present Substance on Enhancing Immunological Response

in Vivo

Sample	No. of Lymphocytes in Spleen (No./mouse)	Uptake of $^3\text{H}$ -thymidine (cpm)
Sample 2	$4.0 \times 10^7 *$	$38,496 \pm 7,270 *$
Sample 1	$4.2 \times 10^7 *$	$40,775 \pm 6,700 *$
Sample 4	$4.1 \times 10^7 *$	$35,226 \pm 8,883 *$
Control	$2.6 \times 10^7$	$23,514 \pm 6,169$

\*,  $P < 0.05$

Example 8:

To  $\text{C}_3\text{H}/\text{He}$  female mice of 9 week old and weighing 21 to 24 g, the present substance prepared in Example 1 were dissolved in water and orally given forcibly at a dose of 5 g/kg body weight and the states of the mice were observed for 3 weeks.

To a control group, only physiological saline was given. The results are given in Table 8. The results show that the groups treated with the present substance exhibit no abnormality and give no death case. Accordingly, it is demonstrated that the present substance is highly safe in use.

Example 9:

To 300 g of the present substance prepared in Example 1 (sample 2), 700 g of milk was added and after being boiled for pasteurization, was maintained at  $43^\circ\text{C}$ . On the other hand, to 20 g of milk which was maintained at  $30^\circ\text{C}$  after being boiled for pasteurization, 2 g of an active lactobacillus containing *Lactobacillus bulgaricus* (manufactured by MEITO NYUGYO, Co.) commercially available was added. The latter solution was added to the former solution containing the present substance and the mixture was maintained at  $43^\circ\text{C}$  for 10 hours with a thermostat and obtained an edible product.

Example 10:

This example relates to one example of formulation of pharmaceutical composition of the present substance.

The present substance (Sample 4) 10 parts by weight  
 Heavy magnesium oxide 15 parts by weight  
 Lactose 75 parts by weight

They were mixed uniformly and made powder or granule to give powdered drug. Further, the powder is put into capsules.

Table 8: Effect on Body Weight Change of the Present Substance

Sample	Body Weight Before Test ( $\bar{X} \pm \text{S.D.}$ )	Body Weight After 3 Weeks ( $\bar{X} \pm \text{S.D.}$ )
Sample 2	$20.7 \pm 0.09$	$23.7 \pm 0.49$
Sample 1	$20.7 \pm 0.95$	$23.4 \pm 0.49$
Sample 3	$20.8 \pm 0.97$	$23.6 \pm 0.50$
Control	$20.8 \pm 0.75$	$23.1 \pm 0.66$

### Claims

1. A dietary fibre obtainable by the extraction of bran with an aqueous solvent, characterised by the following combination of properties:

- (a) an average molecular weight of  $5 \times 10^2$  to  $1 \times 10^5$  measured by the GPC-Lalls method;
- (b) a sugar content as determined by the color reaction of the phenol-sulfuric acid method of 95.3 to 99.0% by weight, and a protein content as determined by the color reaction of the Lowry-Folin method of 0.1 to 3.0% by weight;
- (c) constituent sugars which include at least glucose, xylose and galactose;
- (d) constituent amino acids which include at least aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cysteine, valine, isoleucine, leucine, tyrosine, phenylalanine, hydroxylysine, lysine, histidine, and arginine;
- (e) a molar concentration of the 1.4-glucose bond of 85 to 90% and a molar concentration of the 1.3-glucose bond of 4 to 10%; and
- (f) the following analysis of a constituent sugar component by the Southgate method:

#### % by weight

Water soluble and hardly digestible

polysaccharides	78 to 96
Cellulose	1.0 to 5.7
Hemicellulose	1.7 to 15
Lignin	1.3 to 3.3

2. A dietary fibre obtainable by the extraction of bran with an aqueous solvent, characterised in that it comprises, as principal ingredient, water-soluble and hard-to-digest polysaccharides of which the glucose component has a molar ratio of  $\alpha$ -bound glucose to  $\beta$ -bound glucose of from 1:40 to 1:80.
- 5 3. A process for producing dietary fibre which process comprises extracting bran with an aqueous solvent at a temperature of 170 to 220°C.
4. A process according to claim 3, wherein the temperature is from 180 to 200°C.
- 10 5. A process according to claim 3 or 4 wherein the bran is a wheat bran or a rice bran.
6. A pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as an active principle, a dietary fibre as claimed in claim 1 or 2.
- 15 7. A dietary composition comprising at least one component of a foodstuff or beverage and a dietary fibre as claimed in claim 1 or 2.
8. A dietary fibre as claimed in claim 1 or 2 for use in a method of treatment of the human or animal body by therapy.
- 20 9. A dietary fibre as claimed in claim 1 or 2 for use as an antitumour agent, a stimulant of the growth of *Lactobacillus bifidus* in the intestine, a cholesterol metabolism improving agent or a stimulant of immunological reactions.
- 25 10. Use of a dietary fibre as claimed in claim 1 or 2 in the manufacture of a medicament for use as an antitumour agent, a stimulant of the growth of *Lactobacillus bifidus* in the intestine, a cholesterol metabolism improving agent or a stimulant of immunological reactions.

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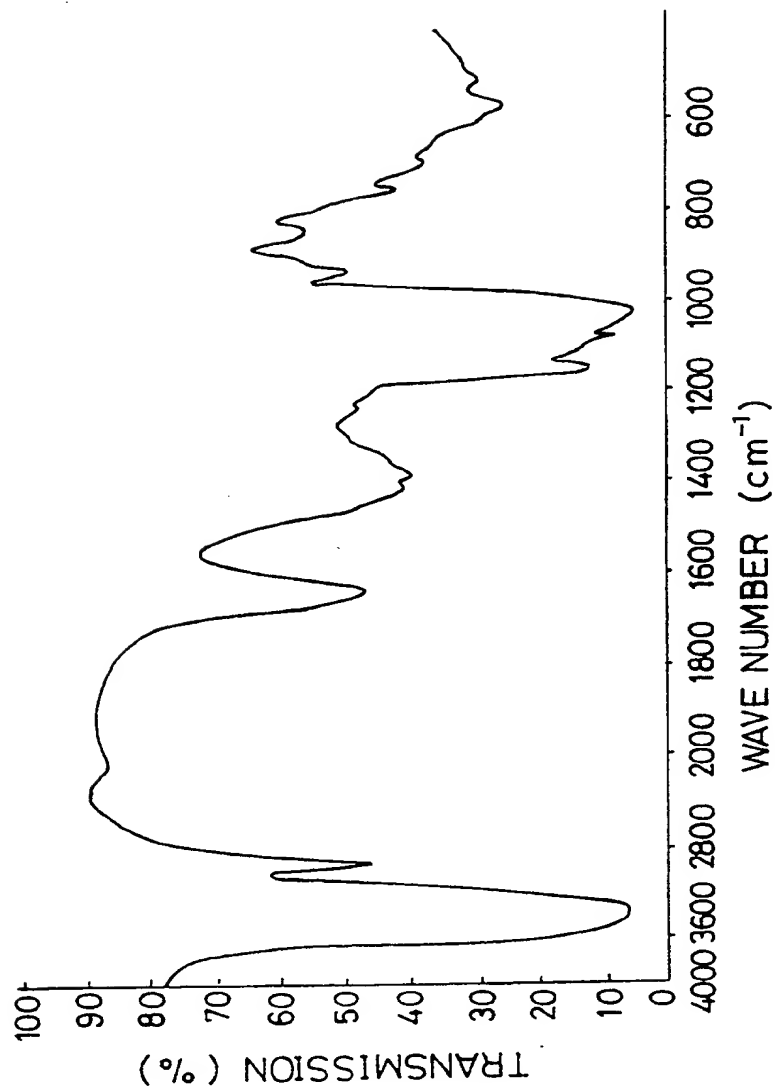
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*Fig. 1*



European Patent  
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# EUROPEAN SEARCH REPORT

Application Number

EP 91 30 4292

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	EP-A-0 172 559 (SAPPORO BREWERIES LTD.) * abstract; claims 1,2 * ---	1,5	A 23 L 1/308 A 61 K 31/715
A	DATABASE WPIL/DERWENT accession no. 86-018080 (03), 1986, Derwent Publications Ltd., London, GB; & JP - A - 60241860 (T. SHIOTA) 30.11.1985 ---	1	
D,A	FR-A-2 335 235 (KUREHA KAGAKU KOGYO K.K.) * claims 1-8 * ---	1	
D,A	PATENT ABSTRACTS OF JAPAN vol. 12, no. 438 (C-544)(3285), 7 November 1988; & JP - A - 63165325 (SNOW BRAND MILK PROD. CO. LTD.) 08.07.1988 -----	1,5	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			A 23 L 1/00 A 61 K 31/00
The present search report has been drawn up for all claims			
Place of search BERLIN		Date of completion of the search 23-07-1991	Examiner SCHULTZE D
<p><b>CATEGORY OF CITED DOCUMENTS</b></p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- &amp; : member of the same patent family, corresponding document</p>			

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